J Mol Cell Cardiol 27, 1213–1218 (1995)

Myocardial Metabolism and Function in Acutely Ischemic and Hypoxemic Isolated Rat Hearts

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(Received 7 July 1994, accepted in revised form 14 November 1994)

M. Samaja, R. Motterlini, S. Allibardi, S. Casalini, G. Merati, A. Corno, and S. Chierchia. Myocardial Metabolism and Function in Acutely Ischemic and Hypoxemic Isolated Rat Hearts. *Journal of Molecular and Cellular Cardiology.* (1995) 27, 1213–1218. We tested the hypothesis that residual oxygen supply during acute low-flow ischaemia or hypoxemia is a major regulator of myocardial performance, metabolism and recovery. Rat hearts were exposed for 20 min to either ischemia (coronary flow reduced to 10% of baseline), hypoxemia (oxygen content reduced to 10% baseline) or a "mixed" condition (combined ischaemia and hypoxemia). The oxygen supply (coronary flow × oxygen content) was matched in all groups ($n=16$ per group). Hypoxemic hearts had the highest performance (systolic and developed pressures, $\pm dP/dt_{max}$ and oxygen uptake) and content of IMP and AMP. Ischaemic hearts had the highest content of ATP, phosphocreatine, adenine nucleotides and purines. As flow and/or oxygenation were restored, post-ischemic hearts showed better functional and metabolic recovery than post-hypoxemic ones. "Mixed" hearts were more similar to hypoxemic ones during oxygen shortage but to ischemic ones during recovery. We conclude that as oxygenation is critically limiting, coronary flow is relatively more important than oxygen supply in determining myocardial function, metabolism and recovery, most likely secondary to changes in the metabolism of diffusible substances.

Key Words: Ischemia; Hypoxemia; Metabolism; ATP; Phosphocreatine; Adenine nucleotides; Recovery.

Introduction

Ischemia is characterized by low coronary flow (CF[1]) with respect to tissue needs. If metabolic factors only are considered, excluding blood-related phenomena, the ischemic dysfunction is driven by low supply of O_2 (QO_2) and low washout of diffusible substances. These phenomena, although superimposed within a single ischaemic episode, are distinct and involve different metabolic patterns. Low QO_2 impairs mitochondrial function and decreases aerobic ATP production (Connett *et al.*, 1990). Washout of diffusible substances such as adenosine, inosine and hypoxanthine depresses the size of the ATP pool (Gutierrez *et al.*, 1988; Soussi *et al.*, 1993; Bak and Ingwall, 1994). Furthermore, since the ATP level during ischemia may be critical in determining dysfunction during recovery (Haas *et al.*, 1984; Takeo *et al.*, 1988; Ambrosio *et al.*, 1989; Rubin *et al.*, 1992), both phenomena are

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[1] List of abbreviations: CF, coronary flow; CPP, coronary perfusion pressure; $+dP/dt_{max}$, maximal rate of contraction; $-dP/dt_{max}$, maximal rate of relaxation; EDP, end-diastolic pressure; H, hypoxemia; HR, heart rate; I, ischaemia; IMP, inosine monophosphate; LVDP, left-ventricle developed pressure; M, "Mixed" case (I+H); PCr, phosphocreatine; PSP, peak systolic pressure; QO_2, supply of O_2; TAN, sum of adenine nucleotides and purines (ATP+ADP+AMP+IMP+adenosine +inosine+hypoxanthine); VO_2, O_2 uptake.

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0022-2828/95/051213+06 $08.00/0

potentially involved during reperfusion injury. However, the relative roles of QO_2 and washout of diffusible substances is still unclear. Separating these two factors may help in understanding the mechanisms underlying ischemic heart disease, myocardial preconditioning and endogenous protection against ischemia.

To gain a better insight into these mechanisms, we recently developed an experimental approach suitable for measurement of the separate effects of reduced QO_2 and reduced CF (Samaja *et al.*, 1994a). For this purpose, hearts were exposed to low-flow ischemia (I) or hypoxemia (H) matched for QO_2 by reducing either CF or PO_2, respectively, to 10% of a reference baseline condition. Care was taken to obtain baseline conditions as similar as possible to the *in vivo* condition and to exert full control of CF and PO_2. Therefore, $QO_2 = CF \times PO_2 \times (O_2$ solubility coefficient). This approach is also suitable to assess what are the major regulators of myocardial performance and metabolism during acute low-flow ischemia or hypoxemia. In fact, if performance is limited by residual QO_2, then a given decrease of QO_2 would produce the same effects regardless of what is reduced, CF or PO_2. On the contrary, if washout of diffusible catabolites is more critical than QO_2, then I and H would induce different metabolic and functional responses. We show that the responses to I and H are different. To better characterize these differences, we designed a "mixed" group (M), where I and H are applied simultaneously at the same QO_2. For most parameters, M hearts do not rank between I and H but rather resemble either I or H. Thus, some of the features normally occurring during low-flow ischaemia seem linked to QO_2, while others are more strictly dependent on CF.

Materials and Methods

Ad libitum-fed Sprague-Dawley male rats (weight 250–280 g) were anesthetized by i.p. heparinized sodium thiopental (10 mg/100 g body weight). Hearts were perfused in a Langendorff mode through the aorta with Krebs-Henseleit buffer containing 2.0 mM free Ca^{2+} and 16.6 mM glucose (pH 7.4, 37°C). The medium was equilibrated at the desired PO_2 in membane oxygenators (Dideco, Mirandola, Italy). PCO_2 was 43 mmHg. A pump delivered the medium at desired flows to a filter (8 μm pore size, 47 mm diameter, Nuclepore Corp., Pleasanton, CA, USA), a preheater and the aortic cannula.

We measured the coronary perfusion pressure (CPP), end diastolic pressure (EDP), peak systolic pressure (PSP), left ventricle-developed pressure (LVDP), heart rate (HR), maximal rate of contraction ($+dP/dt_{max}$) and maximal rate of relaxation ($-dp/dt_{max}$). The effluent was analysed for venous PO_2 (P_vO_2) using an oxygen electrode (YSI mod.5300 Oxygen Monitor, Yellow Springs Inc., OH, USA). The O_2 uptake (VO_2) was calculated from actual P_vO_2, P_aO_2 and CF. Vascular resistance was calculated as (CPP-EDP)/CF/(ventricle weight) (Cunningham *et al.*, 1990).

Hearts were stabilized for 30 min at $CF = 15$ ml/min and $PO_2 = 670$ mmHg ($QO_2 = 14.1$ μmoles/min), with the ventricular balloon volume adjusted to achieve $EDP = 10 \pm 1$ mmHg and kept constant throughout. I and H were applied by reducing either CF to 1.5 ml/min or PO_2 to 67 mmHg. In the "mixed" case (M), $CF = 7.2$ ml/min and $PO_2 = 140$ mmHg. For all three groups, $QO_2 = 1.41$ μmoles/min for 20 min. At end of I, M or H, hearts were reperfused and/or reoxygenated for 20 min.

Baseline measurements were taken at the end of stabilization just before the beginning of O_2 shortage. Other measurements were taken at the end of the 20 min periods of O_2 shortage and recovery. Part of the hearts were freeze-clamped with liquid nitrogen for perchloric acid extraction and tissue dry weight determination at end of baseline, O_2 shortage and recovery. High-pressure liquid chromatography was performed to assay ATP, ADP, AMP, IMP, adenosine, inosine and hypoxanthine (Motterlini *et al.*, 1992). Xanthine and uric acid were never detected by this method.

Data are expressed as mean ±S.E. ANOVA test was performed and if significant, the Fisher's protected least significant difference test was performed to compare the various groups (significance level was $P = 0.05$, two-tailed).

Results

In 90 min control experiments at full QO_2 (not shown), the changes of EDP, LVDP and CPP were less than +1, −5 and +5 mmHg, respectively. As expected, when QO_2 was reduced to 10% of the baseline value, both performance and metabolism were severely depressed (Table 1). However, the dysfunction was critically dependent on how QO_2 was shortened. Diastolic contracture ranked H>M>I, but LVDP, LVDP × HR, $+dP/dt_{max}$ and $-dP/dt_{max}$ ranked H = M>I. Although VO_2 and HR were comparable in all groups (H = M = I), P_vO_2 was much less in H than I hearts. The contents of

Table 1 Data obtained at end of baseline (O_2 supply = 14.1 μmoles/min) and after 20 min of O_2 shortage (ischaemia, "mixed" or hypoxemia) with O_2 supply = 1.41 μmoles/min. Results of ANOVA and Fisher's tests ($P<0.05$): *, Mixed *v* Ischemia; †, Hypoxemia *v* Mixed; ‡, Hypoxemia *v* Ischemia

	Baseline	Ischemia	Mixed	Hypoxemia
n	51	16	17	16
Flow, ml/min	15	1.5	7.2	15
P_aO_2, mmHg	670	670	140	67
QO_2, μmoles/min	14.1	1.41	1.41	1.41
P_vO_2, mmHg	335±31	100±14	10±1*	4±1‡
VO_2, μmoles/min	6.97±0.61	1.20±0.03	1.33±0.01*	1.29±0.02
HR, min^{-1}	270±8	178±12	176±12	173±15
EDP, mmHg	9.3±0.6	5.3±0.4	9.7±1.7	25.6±3.4†‡
LVDP, mmHg	150±5	31±4	49±3*	50±5‡
LVDP × HR, mmHg × 10^3/min	40.6±1.9	5.2±0.5	8.4±0.5*	7.9±0.6‡
$+dP/dt_{max}$, mmHg/s	4955±214	1181±96	2024±88*	2037±139‡
$-dP/dt_{max}$, mmHg/s	3331±130	730±61	1074±53*	1123±100‡
CPP, mmHg	75±4	9±1	33±1*	70±4†‡
Resistance, mmHg × min/ml/g	4.36±0.29	2.15±0.70	3.30±0.24	2.95±0.33
ATP, μmoles/g dw	21.9±1.6	20.4±1.0	18.5±1.3	13.2±0.8†‡
IMP, μmoles/g dw	0.04±0.04	0.15±0.05	0.31±0.04*	0.54±0.06†‡
PCr, μmoles/g dw	26.4±2.4	21.9±1.9	15.6±1.4*	10.4±1.1†‡
TAN, μmoles/g dw	33.9±2.7	33.5±1.5	33.6±1.9	28.6±1.5 †‡

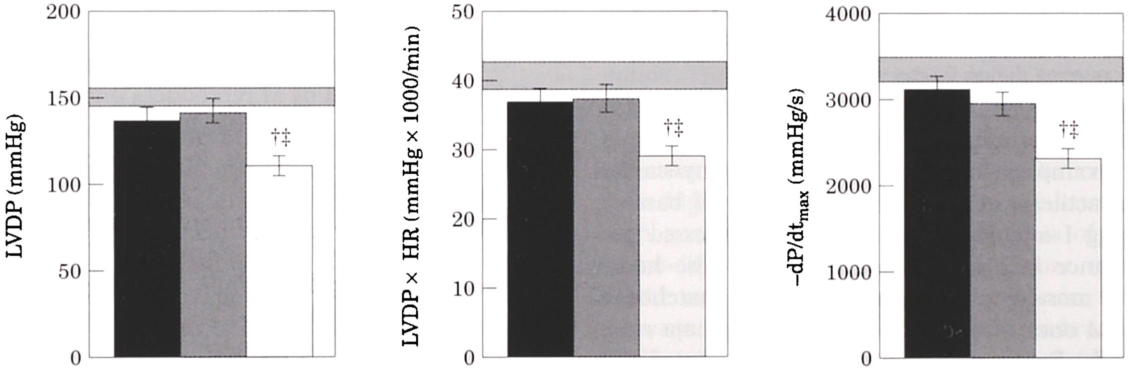

Figure 1 Recovery of mechanical performance 20 min after restoration of flow and/or oxygenation. Results of ANOVA and Fisher's tests ($P<0.05$): *, Mixed (▨) *v* Ischemia (■); †, Hypoxemia (□) *v* Mixed; ‡, Hypoxemia *v* Ischemia. The horizontal strip represents the baseline values (±S.E.).

PCr and ATP were not significantly different from baseline in I hearts, but significantly less in H hearts. The contents of IMP and of the sum of adenine nucleotides and purines (TAN, ATP + ADP + AMP + IMP + adenosine + inosine + hypoxantine) ranked H>M>I and H<M=I, respectively.

Figure 1 shows that the recovery of performance following reoxygenation after H is less than that following reperfusion after I. M hearts behave like I hearts (H<M=I). Both ATP and TAN at recovery ranked H<M=I (Fig. 2). Phosphocreatine was the same in all groups.

Discussion

The QO_2 during baseline compares well with the QO_2 of *in vivo* hearts (8.5–10.1 μmoles/min/g, assuming CF = 70–85 ml/100 g/min, [Hb] = 15.5 g/dl, 98% O_2-saturated at PO_2 = 100 mmHg). The relatively high glucose concentration saturates the glucose transport system (Zweier and Jacobus, 1987) and prevents glucose shortage during I. Indeed, under the lowest CF condition in this study, the glucose supply (190 μmol/min/g dry weight) exceeds by one order of magnitude the maximal glucose utilization by anoxic Langendorff-perfused isolated rat

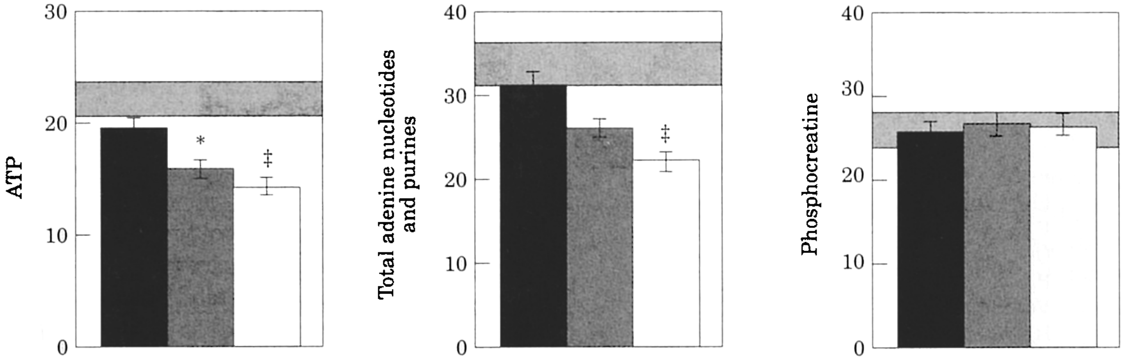

Figure 2 Recovery of metabolic parameters 20 min after restoration of flow and/or oxygenation. Concentrations as μmoles/g d.w. Results of ANOVA and Fisher's tests ($P<0.05$): *, Mixed (▨) *v* Ischemia (■); ‡, Hypoxemia (□) *v* Ischemia. The horizontal strip represents the baseline values (±S.E.).

hearts [14 μmol/min/g dry weight (Rovetto *et al.*, 1975)]. Hyperglycemia does not have any significant effect in our model because the glycolytic rate is regulated by enzyme activity rather than intracellular glucose (Kobayashi and Neely, 1979) and there is no competition between glucose and other substrates.

Performance during O_2 shortage

The dysfunction was different during I, M or H. For example, LVDP × HR, an index of myocardial contractile work, was 13% and 21% of baseline during I and H, respectively. The depressed performance in I hearts indicates that these hearts were more downregulated than QO_2-matched H and M ones as discussed elsewhere (Samaja *et al.*, 1994b). Downregulation during I is associated with low washout of lactate, intracellular lactate retention, lactate-induced acidosis and hence depression of glycolysis (Rovetto *et al.*, 1975; Matthews *et al.*, 1986; Zhou *et al.*, 1991).

In our model, CPP is mainly determined by different CF's under the various experimental conditions, thus CPP ranked H>M>I. Therefore, we can not exclude perfusion heterogeneities especially during I (Hogan *et al.*, 1993). However, resistance did not vary significantly among the various groups (H = M = I) indicating that myocardial perfusion remained essentially constant. This also suggests that the differences observed here are primarily due to metabolic phenomena rather than microcirculatory adjustments.

P_vO_2 was less in H than I hearts, even at the same QO_2, according to observations obtained by others in skeletal muscle (Hogan *et al.*, 1992; Dodd *et al.*, 1993). It is difficult to assess whether this phenomenon is due to non-uniform perfusion secondary to low CPP in I hearts or to lactate-driven downregulation. In both cases, however, residual QO_2 does not appear to be an adequate energy reserve as shown by the essentially unchanged VO_2. We already demonstrated that, as QO_2 is reduced to 1.41 μmoles/min, ATP from anaerobic glycolysis is needed to sustain adequate ATP turnover during both I and H (Samaja *et al.*, 1994b).

The development of diastolic contracture during H but not during I or M reproduces and reinforces previously reported data (Wexler *et al.*, 1986). Despite substantial differences in study design, the explanations for these features are the same: the development of contracture during H may be secondary to impaired resequestration of Ca^{2+} by the sarcoplasmic reticulum while the increased ventricle distensibility during I results from collapsed vasculature and development of acidosis, that decreases the myofibrils sensitivity to $Ca.^{2+}$

Metabolism during O_2 shortage

Measuring the ATP content is limiting with respect to the ATP turnover through creatine kinase but the steady-state content of ATP and PCr may still reflect the overall balance between metabolic supply and utilization because of the rapid turnover rate of their pools (Zweier and Jacobus, 1987). The total ADP content in freeze-clamped tissue is not a valuable index of its activity because ADP is highly compartmentalized in the cell and the free ADP content has to be considered (Humphrey *et al.*, 1990).

In I hearts, the contents of PCr and ATP are relatively higher than in H hearts. This reflects the lower performance and energy demand of I compared to H. The lower IMP, an index of metabolic derangement during O_2 shortage (Achterberg *et al.*, 1988), and higher TAN content during I also reflect the low energy demand in I hearts. This situation allows better ATP/ADP coupling and consequently less degradation of adenylic compounds beyond AMP. On the other hand, in H hearts, high lactate washout releases the inhibition due to acidosis. This leads to high energy demand (Samaja *et al.*, 1994b), reduced ATP/ADP coupling and increased amount of adenine nucleotides degraded beyond AMP. These substances are virtually lost for the ATP pool because some of the degradation reactions, especially that catalysed by 5′-nucleotidase (Bak and Ingwall, 1994), are irreversible (Achterberg *et al.*, 1988). Furthermore, high CF during H removes adenosine, inosine and hypoxanthine (Gutierrez *et al.*, 1988; Soussi *et al.*, 1993; Bak and Ingwall, 1994), contributing to a decrease in TAN content. Failure to detect xanthine and urate in tissue extracts may reflect high xanthine oxidoreductase activity in rat hearts (de Jong *et al.*, 1990) with associated leakage of these substances.

Recovery

The finding that H is more deleterious than I is in agreement with our previous data (Corno *et al.*, Samaja *et al.*, 1994a) and the above considerations. In fact, dysfunction during recovery may be intended as a biological assay of the ability of the heart to restore its high-energy phosphate pool (Bak and Ingwall, 1994). Therefore, better recovery from I than from H reflects better maintenance of the ATP pool during I than during H. In our model, purine salvage is the only way for the heart to restore the ATP pool, because *de novo* purine biosynthesis is slow even when accelerated by O_2 shortage (Zimmer *et al.*, 1973). Phosphocreatine was the same in all groups, because neither creatine nor phosphocreatine are diffusible substances (Savabi, 1988), and thus they do not leak under high-flow conditions like diffusible adenine nucleotides and purines.

Why are M hearts more similar to H ones during the O_2 shortage and to I ones during the reperfusion? Clearly some parameters are more strictly dependent on PO_2 while others depend more strongly on CF. For example, recovery of performance appears to follow a pattern similar to that of ATP and TAN contents during O_2 shortage ($H<M=I$). Thus recovery may depend on the preservation of the adenine nucleotides pool, which is consistent with other observations (Haas *et al.*, 1984; Takeo *et al.*, 1988; Ambrosio *et al.*, 1989; Rubin *et al.*, 1992). As a corollary, since maintenance of the ATP pool depends on the washout of diffusible adenosine, hypoxanthine and inosine, residual CF during low-flow ischemia may appear critical to determine recovery. However, ATP content is reduced in M hearts at the end of recovery despite excellent function possibly indicating that factors other than adenine nucleotides metabolism may be involved in these mechanisms.

Although we fully agree with LH Opie that the concept of critical levels of ATP is now defunct (Opie, 1992), low ATP level is necessarily associated with high amounts of diffusible adenine nucleotides and purines. Therefore, we propose to extend that statement to include flow: if a low ATP situation is associated with high flow, the combination of both factors may ultimately impair the capability of hearts to recover ATP at reperfusion after acute ischemia because of precursors loss.

Conclusion

Ischemia and hypoxemia elicit different metabolic and mechanical responses at equal levels O_2 limitation. Thus, factors different from QO_2 presumably regulate myocardial function and metabolism during ischemia and recovery. The differences between ischemia and hypoxemia appear related to coronary flow: high flows during hypoxemia are associated with greater ATP requirements and loss of membrane-diffusible substances. These factors, as well as the energy imbalance during ischemia, which increase the intracellular level of membrane-diffusible metabolites, need to be investigated as factors responsible for myocardial dysfunction during recovery from acute ischemia.

Acknowledgements

Supported by the Fondazione San Romanello del Monte Tabor, Milano, and the Target Project BTBS, Roma, Italy.

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